

Monatsh Chem (2014) 145:663–673  
DOI 10.1007/s00706-013-1137-x

## ORIGINAL PAPER

# Design, synthesis, antiviral and cytotoxic evaluation of novel acyclic phosphonate nucleotide analogues with a 5,6-dihydro-1*H*-[1,2,3]triazolo[4,5-*d*]pyridazine-4,7-dione system

Emilia Bankowska · Jan Balzarini ·  
Iwona E. Głowacka · Andrzej E. Wróblewski

Received: 30 September 2013 / Accepted: 16 December 2013 / Published online: 25 January 2014  
© The Author(s) 2014. This article is published with open access at Springerlink.com

**Abstract** A series of diethyl 2-(4,5-dimethoxycarbonyl-1*H*-1,2,3-triazol-1-yl)alkylphosphonates was synthesised from  $\omega$ -azidoalkylphosphonates and dimethyl acetylenedicarboxylate and was further transformed into the respective diamides, dihydrazides, and 5,6-dihydro-1*H*-[1,2,3]triazolo[4,5-*d*]pyridazine-4,7-diones as phosphonate analogues of acyclic nucleosides having nucleobases replaced with substituted 1,2,3-triazoles. All compounds containing P–C–C–triazole or P–C–C–CH<sub>2</sub>–triazole moieties exist in single conformations in which the diethoxyphosphoryl and substituted 1,2,3-triazolyl or substituted (1,2,3-triazolyl)methyl groups are oriented *anti*. All phosphonates were evaluated in vitro for activity against a variety of DNA and RNA viruses. None of the compounds were endowed with antiviral activity. They were not cytostatic at 100  $\mu$ M.

**Keywords** Cycloadditions · Cyclizations · Heterocycles · NMR spectroscopy · Conformation

## Introduction

In the past two decades acyclic nucleoside phosphonates (ANPs) have become one of the most important classes of antiviral drugs [1]. Three of them (adefovir, cidofovir, tenofovir; Fig. 1) have been marketed for treatment of viral infection caused by HIV, HBV, HSV and other DNA viruses [2–5]. The concept of acyclic nucleosides is based on the assumption that an acyclic moiety most often bearing an oxygen atom mimics the furanose ring at least partially. Acyclic nucleoside phosphonates require conversion in vivo to their triphosphate metabolites to become active [2, 6]. The replacement of the natural phosphate moiety by a phosphonate group makes analogues less susceptible to enzymatic hydrolysis [7, 8].

Further studies in this field brought a new generation of nucleotide/nucleoside analogues in which natural nucleobases were modified as exemplified by the 2,4-diaminopyrimidine framework present in antiviral HPMPO-DAPy and PMPO-DAPy [9–11] and the 1,2,4-triazole ring in ribavirin (Fig. 2) [12].

The antiviral activity of ribavirin stimulated interest in replacing the 1,2,4-triazole system with an isomeric 1,2,3-triazole ring because several 1,2,3-triazoles exhibit antibacterial [13–15], antifungal [15–17], anticancer [18–20], anti-inflammatory [21, 22] and antiviral [23–26] properties. It was found that carbocyclic analogues **1** and phosphonocarbocyclic analogues **2** of ribavirin displayed antiviral activity against HIV-1 (Fig. 3) [27].

Furthermore, preliminary structure–activity relationship evaluation of 1,2,3-triazole nucleoside phosphonates **3** and **4** suggested that this scaffold could be further optimised to afford selective inhibitors of HCV replication (Fig. 4) [28].

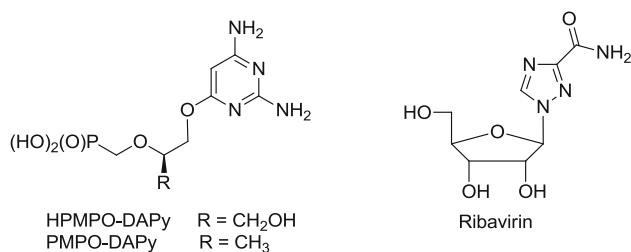
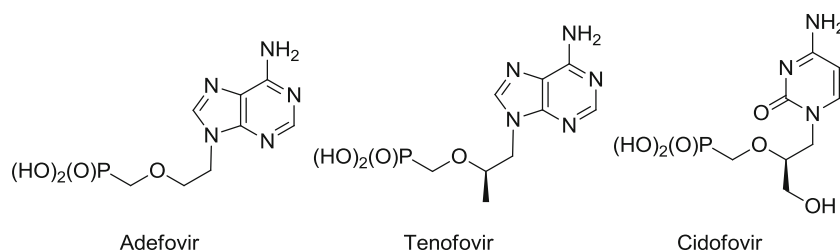
On the other hand, it was reported that nucleoside analogues **5** and **6** containing the 5,6-dihydro-1*H*-imidazo[4,5-

**Electronic supplementary material** The online version of this article (doi:10.1007/s00706-013-1137-x) contains supplementary material, which is available to authorized users.

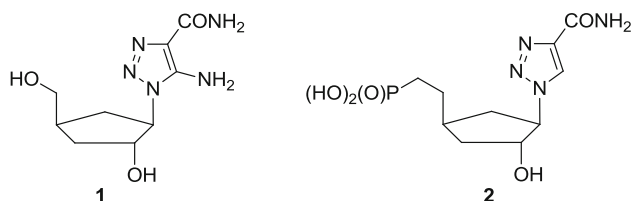
E. Bankowska · I. E. Głowacka · A. E. Wróblewski (✉)  
Bioorganic Chemistry Laboratory, Faculty of Pharmacy,  
Medical University of Łódź, Muszyńskiego 1, 90-151 Łódź,  
Poland  
e-mail: andrzej.wroblewski@umed.lodz.pl

J. Balzarini  
Rega Institute for Medical Research, KU Leuven,  
Minderbroedersstraat 10, 3000 Leuven, Belgium

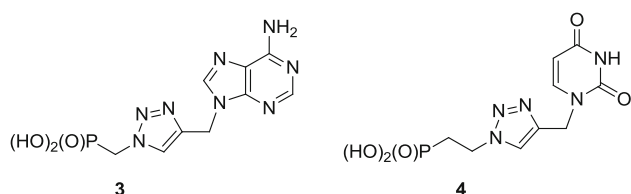
**Fig. 1** ANPs marketed for treatment of viral infections



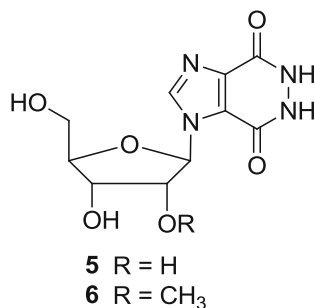
**Fig. 2** Structures of HPMPO-DAPy, PMPO-DAPy and ribavirin



**Fig. 3** Antiviral analogues of ribavirin having the 1,2,3-triazole ring



**Fig. 4** 1,2,3-Triazole nucleoside phosphonates as potential inhibitors of HCV replication



**Fig. 5** Cyclic nucleoside analogues based on the 5,6-dihydro-1H-imidazo[4,5-d]pyridazine-4,7-dione framework

*d*]pyridazine-4,7-dione ring instead of the natural purine framework exhibited antiviral activity by inhibition of a viral helicase from West Nile Virus (WNV) and HCV (Fig. 5). These observations may be useful in designing a lead structure for the development of new classes of antiviral agents [29].

Based on the active compounds already discussed, a novel series of phosphonate analogues **11** having the 5,6-dihydro-1*H*-[1,2,3]triazolo[4,5-*d*]pyridazine-4,7-dione system was designed as potential antiviral agents (Scheme 1). Furthermore, because their immediate precursor dihydrazides **10** as well as diamides **9** share several common structural features with ribavirin, they also may show antiviral activity. The key step of our synthetic plan involves the 1,3-dipolar cycloaddition of dimethyl acetylenedicarboxylate and  $\omega$ -azidoalkylphosphonates **7** which contain structurally diversified alkyl chains to provide the intermediate diesters **8**.

## Results and discussion

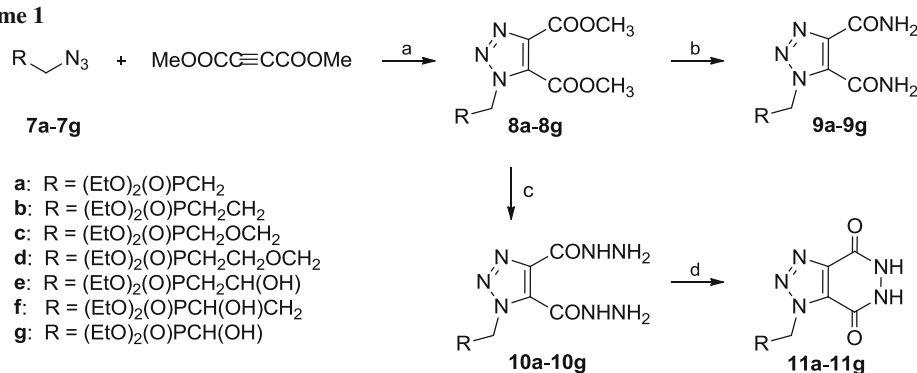
### Chemistry

The 1,2,3-triazoles **8a–8d** and **8f** were synthesised in 64–98 % yield employing the Huisgen 1,3-dipolar cycloaddition of the corresponding azidophosphonates **7** and dimethyl acetylenedicarboxylate at 110 °C in the same way as the known compounds **8e** and **8g** [30, 31]. They were finally purified either by chromatography on a silica gel column or by crystallisation (Scheme 1).

The required azidoalkylphosphonates **7a–7e** and **7g** have already been described in the literature [30, 32–36]. Azidophosphonate **7f** was obtained in the Abramov reaction [37, 38] from 3-azidopropanal [39] and diethyl phosphite in 34 % yield (Scheme 2). It was found that 3-azidopropanal is unstable in the presence of triethylamine used as a catalyst in this reaction. For this reason only 0.4 equiv. of diethyl phosphite was applied to avoid tedious separation of **7f** from the reaction mixture.

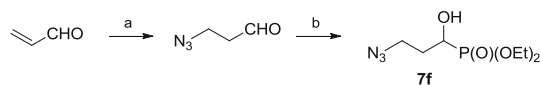
The diamides **9a–9g** were obtained from diesters **8a–8g** by ammonolysis according to a standard protocol (Scheme 1) [6, 40]. The crude products were subjected to column chromatography on silica gel and finally purified

Scheme 1

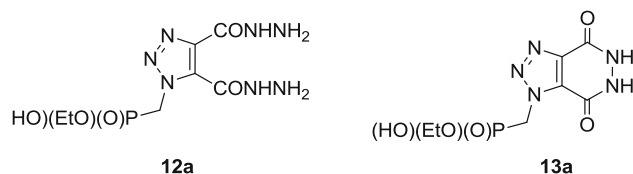


Reagents and conditions: a. toluene, 110 °C, 4 h; b. aqueous ammonia, EtOH, rt, 24 h; c. hydrazine hydrate, EtOH, reflux, 2 h; d. 10% HCl, 90 °C, 2.5 h.

Scheme 2



Reagents and conditions: a.  $\text{NaN}_3$ , acetic acid, -10 °C, 2 h; b.  $(\text{EtO})_2\text{P}(\text{O})\text{H}$ ,  $\text{NEt}_3$ .

Fig. 6 Compounds **12a** and **13a**

by crystallisation to give **9a-9g** in 37–61 % yields. The  $^1\text{H}$  NMR spectra of diamides **9a-9g** in chloroform-*d* confirmed the nonequivalence of protons from the carbamoyl groups because in the 6.0–10.8 ppm region four broad singlets were always observed.

The diesters **8a-8g** were also converted to the corresponding dihydrazides **10a-10g** with hydrazine hydrate (Scheme 1). Preliminary attempts at synthesising dihydrazide **10a** followed the literature procedure [41] and showed that refluxing phosphonate **8a** and hydrazine hydrate in ethanolic solution for 5 h led to the formation of several products. The  $^{31}\text{P}$  NMR spectrum of the reaction mixture revealed the presence of the expected phosphonate **10a** (76 %), bicyclic 1,2,3-triazolopyridazinedione **11a** (11 %) and their monodealkylated counterparts **12a** and **13a** (10 and 3 %, respectively) (Fig. 6). When hydrazinolysis of the diester **8a** was conducted for 2 h only phosphonates **10a** (79 %) and **11a** (21 %) were produced. For this reason syntheses of dihydrazides **10b-10g** were performed under

the same conditions. However, it appeared that for compounds **8c** and **8g** significant dealkylation still occurred. In both cases the respective crude reaction mixtures contained almost 50 % of by-products. Purifications on silica gel columns and crystallisations gave dihydrazides **10a-10g** in 28–66 % yields.

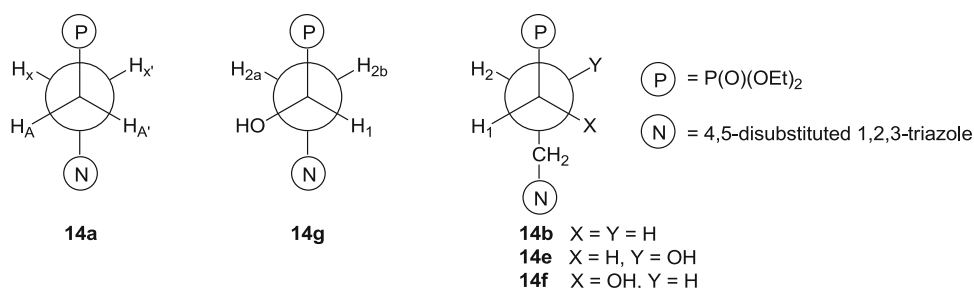
Finally, heating dihydrazides **10a-10g** with 10 % hydrochloric acid for 2.5 h gave [1,2,3]triazolo[4,5-*d*]pyridazine-4,7-diones **11a-11g** (Scheme 1) in 30–66 % yields [41].

### Conformational analysis

Detailed analyses of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data revealed conformational preferences of phosphonates described in this paper. Compounds **8a-11a** contain an 1,2-substituted ethylene fragment which does not freely rotate around a C–C bond because their  $^1\text{H}$  NMR spectra display AA'XX'P patterns. A similar spectrum was also noticed for 2-azidoethylphosphonate [31]. Antiperiplanar disposition of the diethoxyphosphoryl groups and substituted 1,2,3-triazoles **14a** (Fig. 7) was proved by the presence of two identical  $^3J(\text{P}-\text{H}_X) = 10.5$  Hz couplings which were calculated from the  $^1\text{H}\{^{31}\text{P}\}$  NMR spectrum of **8a**.

In the  $^1\text{H}$  NMR spectra of compounds **8g**, **10g**, and **11g** three hydrogen atoms attached to the two-carbon linker appeared as deceptively simple but very similar ABXP spectral patterns. However, the relevant  $^3J(\text{H1}-\text{H2a})$  and  $^3J(\text{H1}-\text{H2b})$  coupling constants were precisely calculated (10.2 and 3.6 Hz, respectively) from the  $^1\text{H}$  NMR spectrum of diamide **9g**. These values [42] together with small couplings for  $^3J(\text{P}-\text{H2a})$  and  $^3J(\text{P}-\text{H2b})$  [43, 44] allowed us to unequivocally establish **14g** (Fig. 7) as the preferred conformation of phosphonate **9g** although other phosphonates from this series very likely adopt the same *anti* conformation.

**Fig. 7** Preferred conformations of the phosphonates described in this study



Large values (16.9–19.2 Hz) of  $^3J(\text{P-CC-C3})$  [44–46] observed in the  $^{13}\text{C}$  NMR spectra of phosphonates **8–11** containing a three-carbon fragment between the phosphorus atom and the 1,2,3-triazole ring (series **b**, **e**, and **f**) evidenced the preference of antiperiplanar conformations **14b**, **14e**, and **14f** (Fig. 7) for these compounds. This conclusion was further supported by vicinal H1–H2 couplings calculated for 2- and 1-hydroxyphosphonates (series **e** and **f**) which clearly indicated *gauche* (3.0–3.6 Hz) and *anti* (9.6–10.8 Hz) arrangements of the respective H–C1C2–H protons.

Although values (9.3–12.1 Hz) of  $^3J(\text{P-C-O-C})$  were easily extracted from the  $^{13}\text{C}$  NMR spectra of phosphonates **8–11** (series **c**) they could not be unequivocally applied in the estimation of the extent to which rotation around the PC–OC bond is hindered because the angular dependence of  $^3J(\text{P-C-O-C})$  has not been established so far. However, the rotation around the OC–CN bond is not restricted because vicinal H<sub>2</sub>C–CH<sub>2</sub> coupling constants observed for phosphonates **8c–11c** fall in the 4.8–5.2 Hz range. On the other hand, on the basis of the values of  $^3J(\text{HC-CH})$  found for PH<sub>2</sub>C–CH<sub>2</sub>O and OH<sub>2</sub>C–CH<sub>2</sub>N units (7.2–7.8 and 5.1–5.7 Hz, respectively), full conformational freedom within a five-atom linker in phosphonates **8–11** (series **d**) is anticipated.

#### Antiviral activity evaluation

The synthesised compounds **9a–9g**, **10a–10g**, and **11a–11g** were evaluated for their antiviral activities against a wide variety of DNA and RNA viruses using the following cell-based assays: (a) human embryonic lung (HEL) cell: herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), herpes simplex virus-1 (TK<sup>−</sup> ACV<sup>T</sup> KOS), vaccinia virus, vesicular stomatitis virus, varicella-zoster virus (TK<sup>+</sup> VZV strain OKA and TK VZV strain 07-1) and cytomegalovirus (CMV) (strain AD-169 and Davis); (b) CEM cell cultures: human immunodeficiency virus-1 (HIV-1) and HIV-2; (c) Vero cell cultures: para-influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus; (d) HeLa cell cultures: vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus; (e) Crandel-Rees feline kidney (CRFK) cell cultures: feline corona virus

(FIPV) and feline herpes virus (FHV) and (f) Madin Darby Canine kidney (MDCK) cell culture: influenza A virus H1N1 subtype A/PR/8, influenza H3N2 subtype A/HK/7/87 and influenza B virus (B/HK/5/72). Ganciclovir, cidofovir, acyclovir, brivudin, (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA], ribavirin, oseltamivir carboxylate, amantadine and rimantadine were used as the reference compounds. The antiviral activity was expressed as the EC<sub>50</sub>: the compound concentration required to reduce virus plaques formation (VZV, CMV) by 50 % or to reduce virus-induced cytopathogenicity by 50 % (other viruses). None of the compounds showed appreciable antiviral activity at sub-toxic concentrations.

#### Evaluation of cytotoxicity

The cytotoxicity of the tested compounds towards the uninfected host cells was defined as the minimum cytotoxic concentration (MMC) that causes a microscopically detectable alteration of normal cell morphology. The 50 % cytotoxic concentration (CC<sub>50</sub>), i.e. causing a 50 % decrease in cell viability, was determined using a colorimetric 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay system. None of the tested compounds affected the cell morphology of Vero, HEL, HeLa or CRFK cells (MCC or CC<sub>50</sub>) at compound concentrations up to 100 μM. They were also not cytostatic against murine leukemia and human CEM and HeLa cells at 100 μM.

#### Conclusions

Phosphonylated 1,2,3-triazoles **8a–8g** were obtained in good to excellent yields by 1,3-dipolar cycloaddition between  $\omega$ -azidophosphonates **7a–7g** and dimethyl acetylenedicarboxylate. New series of diamides **9a–9g**, dihydrazides **10a–10g** and 1,2,3-triazolopyridazinediones **11a–11g** were efficiently synthesised as phosphonate analogues of acyclic nucleosides in which nucleobases were replaced by substituted 1,2,3-triazoles.

Compounds **8–11** (series **a, b, e, f, g**) exist in single conformations in which diethoxyphosphoryl and substituted 1,2,3-triazolyl (series **a** and **b**) or (1,2,3-triazolyl)methyl groups (series **e, f, g**) prefer the *anti* orientation.

All synthesised compounds were evaluated for their antiviral activity against DNA and RNA viruses and were inactive. None of the compounds were cytotoxic (Vero, HEL, HeLa) or cytostatic (L1210, CEM, HeLa) at a concentration up to 100  $\mu$ M.

## Experimental

The  $^1\text{H}$  NMR spectra were recorded in  $\text{CDCl}_3$ ,  $\text{CD}_3\text{OD}$  or  $\text{D}_2\text{O}$  on the following spectrometers: Varian Mercury-300 and Bruker Avance III (600 MHz) with TMS as an internal standard; chemical shifts  $\delta$  in ppm with respect to TMS; coupling constants  $J$  in Hz. The  $^{13}\text{C}$  NMR spectra were recorded for  $\text{CDCl}_3$ ,  $\text{CD}_3\text{OD}$  or  $\text{D}_2\text{O}$  solutions on Varian Mercury-300 and Bruker Avance III (600 MHz) machines at 75.5 and 150.5 MHz, respectively. The  $^{31}\text{P}$  NMR spectra were recorded in  $\text{CDCl}_3$ ,  $\text{CD}_3\text{OD}$  or  $\text{D}_2\text{O}$  on Varian Mercury-300 and Bruker Avance III (600 MHz) spectrometers at 121.5 and 243 MHz, respectively.

IR spectra were measured on an Infinity MI-60 FT-IR spectrometer. Melting points were determined on the Boetius apparatus. Elemental analyses were performed by the microanalytical laboratory of the host institution on Perkin Elmer PE 2400 CHNS analyzer and the results were found to be in good agreement ( $\pm 0.3\%$ ) with the calculated values.

The following absorbents were used: column chromatography, Merck silica gel 60 (70–230 mesh); analytical TLC, Merck TLC plastic sheets, silica gel 60 F<sub>254</sub>. TLC plates were developed in chloroform–methanol solvent systems. Visualization of spots was effected with iodine vapours. All solvents were dried according to standard literature methods.

### Diethyl 3-azido-1-hydroxypropylphosphonate (**7f**, $\text{C}_7\text{H}_{16}\text{N}_3\text{O}_4\text{P}$ )

A mixture of 5.77 g 3-azidopropanal [39] (0.0582 mol), 3.0  $\text{cm}^3$  diethyl phosphite (0.023 mol) and 0.81  $\text{cm}^3$  triethylamine (0.0058 mol) was stirred at 5  $^\circ\text{C}$  for 24 h. The crude product was subjected to chromatography on silica gel with chloroform/methanol (100:1 and 50:1, v/v) to give a yellow oil (4.671 g, 34 %). IR (film):  $\bar{\nu} = 3,272, 2,985, 2,934, 2,911, 2,874, 2,102, 1,227, 1,028\text{ cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta = 1.35$  (t,  $J = 7.1$  Hz, 6H,  $2 \times \text{POCH}_2\text{CH}_3$ ), 1.87–2.06 (m, 3H,  $\text{CH}_2$ , OH), 3.55 (t,  $J = 6.3$  Hz, 2H,  $\text{CH}_2\text{N}_3$ ), 4.01 (dt,  $J = 9.8, 4.6$  Hz, 1H, H-1), 4.12–4.24 (m, 4H,  $2 \times \text{POCH}_2\text{CH}_3$ ) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75.5 MHz):  $\delta = 16.6$  and 16.7 (2 d,  $J = 5.4$  Hz, POCC), 30.8 (d,

$J = 2.6$  Hz, C-2), 47.7 (d,  $J = 15.5$  Hz, C-3), 62.9 and 63.2 (2 d,  $J = 7.2$  Hz, POC), 64.6 (d,  $J = 164.6$  Hz, C-1) ppm;  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 121.5 MHz):  $\delta = 25.28$  ppm.

### Synthesis of 1,2,3-triazoles **8a–8g** (general procedure)

A solution of azidophosphonate **7a–7g** (1.00 mmol) and dimethyl acetylenedicarboxylate **13** (1.00 mmol) in 4  $\text{cm}^3$  toluene was refluxed for 4 h. The reaction mixtures were concentrated to dryness to leave yellow oils which were purified on silica gel columns with chloroform/methanol (100:1, v/v) or were crystallised to give 1,2,3-triazoles **8a–8g**.

### Diethyl 2-(4,5-dimethoxycarbonyl-1H-1,2,3-triazol-1-yl)ethylphosphonate (**8a**, $\text{C}_{12}\text{H}_{20}\text{N}_3\text{O}_7\text{P}$ )

From 0.450 g azidophosphonate **7a** (2.17 mmol) and 0.309 g dimethyl acetylenedicarboxylate **13** (2.17 mmol) phosphonate **8a** was obtained as a yellowish oil (0.674 g, 89 %) after chromatography on a silica gel column with chloroform/methanol (100:1, v/v). IR (film):  $\bar{\nu} = 3,462, 2,985, 2,958, 1,736, 1,468, 1,447, 1,225, 1,140, 1,060, 1,025, 957\text{ cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta = 1.33$  (t,  $J = 7.1$  Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 2.40–2.52 (m, 2H,  $\text{PCH}_2$ ), 3.98 (s, 3H,  $\text{OCH}_3$ ), 4.02 (s, 3H,  $\text{OCH}_3$ ), 4.08–4.18 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 4.82–4.91 (m, 2H,  $\text{CH}_2\text{N}$ ) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta = 16.6$  (d,  $J = 5.7$  Hz, CCOP), 27.2 (d,  $J = 140.6$  Hz, PC), 45.4 (s, PCCN), 52.9 (s,  $\text{OCH}_3$ ), 53.7 (s,  $\text{OCH}_3$ ), 62.4 (d,  $J = 6.3$  Hz, CCOP), 129.8 and 140.2 (2 s, C=C), 158.6 and 160.4 (2 s, C=O) ppm;  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 121 MHz):  $\delta = 25.9$  ppm.

### Diethyl 3-(4,5-dimethoxycarbonyl-1H-1,2,3-triazol-1-yl)propylphosphonate (**8b**, $\text{C}_{13}\text{H}_{22}\text{N}_3\text{O}_7\text{P}$ )

From 0.785 g azidophosphonate **7b** (3.565 mmol) and 0.507 g dimethyl acetylenedicarboxylate **13** (3.565 mmol) phosphonate **8b** was obtained as a yellowish oil (1.067 g, 83 %) after chromatography on a silica gel column with chloroform/methanol (100:1, v/v). IR (film):  $\bar{\nu} = 2,953, 2,836, 1,736, 1,463, 1,226, 1,030\text{ cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta = 1.34$  (t,  $J = 7.1$  Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 1.72–1.84 (m, 2H,  $\text{PCH}_2\text{CH}_2$ ), 2.16–2.31 (m, 2H,  $\text{PCH}_2$ ), 4.00 (s, 3H,  $\text{OCH}_3$ ), 4.03 (s, 3H,  $\text{OCH}_3$ ), 4.05–4.18 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 4.71 (t,  $J = 7.1$  Hz, 2H,  $\text{CH}_2\text{N}$ ) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta = 16.5$  (d,  $J = 6.0$  Hz, POCC), 22.6 (d,  $J = 143.1$  Hz, PC), 23.5 (d,  $J = 4.3$  Hz, PCC), 50.4 (d,  $J = 16.9$  Hz, PCCC), 52.7 (s,  $\text{OCH}_3$ ), 53.2 (s,  $\text{OCH}_3$ ), 61.8 (d,  $J = 6.3$  Hz, POC), 129.7 and 139.8 (2 s, C=C), 158.6 and 160.3 (2 s, C=O) ppm;  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 121 MHz):  $\delta = 30.7$  ppm.

### Diethyl 2-(4,5-dimethoxycarbonyl-1H-1,2,3-triazol-1-yl)ethoxymethylphosphonate (**8c**, $\text{C}_{13}\text{H}_{22}\text{N}_3\text{O}_8\text{P}$ )

From 1.027 g azidophosphonate **7c** (4.330 mmol) and 0.615 g dimethyl acetylenedicarboxylate **13** (4.330 mmol) phosphonate **8c** was obtained as a yellowish oil (1.538 g, 93 %) after chromatography on a silica gel column with chloroform/



methanol (100:1, v/v). IR (film):  $\bar{\nu}$  = 3,459, 2,983, 2,957, 2,908, 1,732, 1,462, 1,225, 1,117, 1,060, 1,027, 960  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz):  $\delta$  = 1.31 (t,  $J$  = 7.0 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 3.74 (d,  $J$  = 7.9 Hz, 2H,  $\text{PCH}_2$ ), 3.98 (s, 3H,  $\text{OCH}_3$ ), 4.02 (s, 3H,  $\text{OCH}_3$ ), 4.02 (t,  $J$  = 5.2 Hz, 2H,  $\text{OCH}_2\text{CH}_2\text{N}$ ), 4.08–4.13 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 4.86 (t,  $J$  = 5.2 Hz, 2H,  $\text{OCH}_2\text{CH}_2\text{N}$ ) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  = 16.5 (d,  $J$  = 5.7 Hz,  $\text{POCC}$ ), 49.8 (s, CN), 52.8 (s,  $\text{OCH}_3$ ), 53.5 (s,  $\text{OCH}_3$ ), 62.6 (d,  $J$  = 6.5 Hz,  $\text{POCC}$ ), 65.2 (d,  $J$  = 164.7 Hz, PC), 71.0 (d,  $J$  = 9.3 Hz,  $\text{PCOC}$ ), 131.0 and 139.6 (2 s, C=C), 158.8 and 160.3 (2 s, C=O) ppm;  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 243 MHz):  $\delta$  = 20.04 ppm.

*Diethyl 2-[2-(4,5-dimethoxycarbonyl-1H-1,2,3-triazol-1-yl)-ethoxy]ethylphosphonate (8d,  $\text{C}_{14}\text{H}_{24}\text{N}_3\text{O}_8\text{P}$ )*

From 0.647 g azidophosphonate **7d** (2.68 mmol) and 0.382 g dimethyl acetylenedicarboxylate **13** (2.68 mmol) phosphonate **8d** (1.030 g, 98 %) was obtained as a yellowish oil. The crude product was sufficiently pure and was used in the next step without further purification. IR (film):  $\bar{\nu}$  = 3,459, 2,983, 2,957, 2,909, 1,736, 1,466, 1,229, 1,118, 1,063, 1,027, 963  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 600 MHz):  $\delta$  = 1.32 (t,  $J$  = 7.1 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 2.00 (dt,  $J$  = 15.3, 7.7 Hz, 2H,  $\text{PCH}_2$ ), 3.63 (dt,  $J$  = 10.4, 7.7 Hz, 2H,  $\text{PCH}_2\text{CH}_2\text{O}$ ), 3.82 (t,  $J$  = 5.2 Hz, 2H,  $\text{OCH}_2\text{CH}_2\text{N}$ ), 4.00 (s, 3H,  $\text{OCH}_3$ ), 4.01 (s, 3H,  $\text{OCH}_3$ ), 4.05–4.12 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 4.82 (t,  $J$  = 5.2 Hz, 2H,  $\text{OCH}_2\text{CH}_2\text{N}$ ) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 150 MHz):  $\delta$  = 16.3 (d,  $J$  = 5.7 Hz,  $\text{POCC}$ ), 26.7 (d,  $J$  = 139.3 Hz, PC), 49.8 (s,  $\text{OCCN}$ ), 52.6 (s,  $\text{OCH}_3$ ), 53.3 (s,  $\text{OCH}_3$ ), 61.6 (d,  $J$  = 6.4 Hz,  $\text{CCOP}$ ), 65.3 (s, PCC), 68.7 (s,  $\text{OCCN}$ ), 131.4 and 139.5 (2 s, C=C), 159.1 and 160.3 (2 s, C=O) ppm;  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 243 MHz):  $\delta$  = 27.58 ppm.

*Diethyl 1-hydroxy-3-(4,5-dimethoxycarbonyl-1H-1,2,3-triazol-1-yl)propylphosphonate (8f,  $\text{C}_{13}\text{H}_{22}\text{N}_3\text{O}_8\text{P}$ )*

From 0.560 g azidophosphonate **7f** (2.36 mmol) and 0.335 g dimethyl acetylenedicarboxylate **13** (2.36 mmol) phosphonate **8f** (0.850 g, 95 %) was obtained as a white amorphous solid after chromatography on a silica gel column with chloroform/methanol (100:1, v/v). M.p.: 107–109 °C; IR (film):  $\bar{\nu}$  = 3,420, 3,218, 3,081, 2,985, 2,878, 1,675, 1,451, 1,280, 1,223, 1,030, 970  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  = 1.33 and 1.34 (2 t,  $J$  = 7.2 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 2.28–2.47 (m, 2H,  $\text{PCCCH}_2$ ), 3.70 (dd,  $J$  = 6.0, 5.1 Hz, 1H, OH), 3.84 (dddd,  $J$  = 10.5, 6.3, 6.0, 3.3 Hz, 1H, PCH), 3.98 (s,  $\text{OCH}_3$ ), 4.00 (s,  $\text{OCH}_3$ ), 4.10–4.22 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 4.75–4.90 (m, 2H,  $\text{CH}_2\text{N}$ ) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  = 16.6 and 16.7 (2 d,  $J$  = 5.4 Hz,  $\text{CCOP}$ ), 31.9 (d,  $J$  = 3.2 Hz, PCC), 47.2 (d,  $J$  = 16.3 Hz, PCCC), 52.8 (s,  $\text{OCH}_3$ ), 53.6 (s,  $\text{OCH}_3$ ), 63.0 and 63.3 (2 d,  $J$  = 7.3 Hz,  $\text{CCOP}$ ), 64.6 (d,  $J$  = 164.2 Hz, PC), 130.3 and 139.7 (2 s, C=C), 158.8 and 160.4 (2 s, C=O) ppm;  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 121 MHz):  $\delta$  = 23.5 ppm.

*Synthesis of diamides 9a–9g (general procedure)*

To a solution of the diester **8a–8g** (1.00 mmol) in 14  $\text{cm}^3$  ethanol 16  $\text{cm}^3$  was added aqueous ammonia. The reaction mixtures were stirred at room temperature for 24 h. Ethanol and excess ammonia were evaporated in vacuo. Diamides **9a–9g** were purified on silica gel columns with chloroform/methanol or by crystallisation.

*Diethyl 2-(4,5-dicarbamoyl-1H-1,2,3-triazol-1-yl)ethylphosphonate (9a,  $\text{C}_{10}\text{H}_{18}\text{N}_5\text{O}_5\text{P}$ )*

From 0.165 g diester **8a** (0.472 mmol) diamide **9a** was obtained as a white amorphous solid (0.065 g, 42 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, v/v) followed by crystallisation from ethanol/diethyl ether. M.p.: 172–173 °C; IR (KBr):  $\bar{\nu}$  = 3,428, 3,228, 3,113, 2,986, 2,932, 1,687, 1,451, 1,221, 1,023  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  = 1.34 (t,  $J$  = 6.9 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 2.41–2.53 (m, 2H,  $\text{PCH}_2$ ), 4.09–4.21 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 5.08–5.17 (m, 2H,  $\text{CH}_2\text{N}$ ), 6.01 (s, 2H,  $\text{NH}_2$ ), 7.58 (s, 1H, NH), 10.77 (s, 1H, NH) ppm;  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 75 MHz):  $\delta$  = 16.8 (d,  $J$  = 6.0 Hz,  $\text{CCOP}$ ), 27.3 (d,  $J$  = 140.3 Hz, PC), 47.2 (s,  $\text{PCCN}$ ), 63.8 (d,  $J$  = 6.6 Hz,  $\text{CCOP}$ ), 132.1 and 140.4 (2 s, C=C), 160.3 and 165.2 (2 s, C=O) ppm;  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 121 MHz):  $\delta$  = 26.5 ppm.

*Diethyl 3-(4,5-dicarbamoyl-1H-1,2,3-triazol-1-yl)propylphosphonate (9b,  $\text{C}_{11}\text{H}_{20}\text{N}_5\text{O}_5\text{P}$ )*

From 0.114 g diester **8b** (0.314 mmol) diamide **9b** was obtained as a white amorphous solid (0.070 g, 61 %) after chromatography on a silica gel column with chloroform/methanol (50:1, v/v) and crystallisation from ethanol. M.p.: 143–144 °C; IR (KBr):  $\bar{\nu}$  = 3,455, 3,270, 2,984, 2,909, 1,694, 1,629, 1,592, 1,454, 1,205, 1,018, 961  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  = 1.31 (t,  $J$  = 6.9 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 1.74–1.86 (m, 2H,  $\text{PCH}_2\text{CH}_2$ ), 2.18–2.32 (m, 2H,  $\text{PCH}_2$ ), 4.04–4.16 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 4.98 (t,  $J$  = 6.9 Hz, 2H,  $\text{CH}_2\text{N}$ ), 6.13 (s, 1H, NH), 6.25 (s, 1H, NH), 7.62 (s, 1H, NH), 10.79 (s, 1H, NH) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  = 16.7 (d,  $J$  = 6.6 Hz,  $\text{CCOP}$ ), 23.0 (d,  $J$  = 141.9 Hz, PC), 23.8 (d,  $J$  = 4.3 Hz, PCC), 51.8 (d,  $J$  = 19.2 Hz, PCCC), 62.0 (d,  $J$  = 6.6 Hz,  $\text{CCOP}$ ), 130.7 and 138.7 (2 s, C=C), 158.6 and 163.7 (2 s, C=O) ppm;  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 121 MHz):  $\delta$  = 31.4 ppm.

*Diethyl [2-(4,5-dicarbamoyl-1H-1,2,3-triazol-1-yl)ethoxy]-methylphosphonate (9c,  $\text{C}_{11}\text{H}_{20}\text{N}_5\text{O}_6\text{P}$ )*

From 0.280 g diester **8c** (0.738 mmol) diamide **9c** was obtained as a white amorphous solid (0.139 g, 54 %) after chromatography on a silica gel column with chloroform/methanol (50:1, v/v). M.p.: 100–101 °C; IR (KBr):  $\bar{\nu}$  = 3,361, 3,259, 3,110, 2,990, 2,959, 2,901, 1,689, 1,613, 1,454, 1,303, 1,219, 1,118, 1,048, 1,019, 967,

943  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 600 MHz):  $\delta$  = 1.29 (t,  $J$  = 7.1 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 3.89 (d,  $J$  = 8.5 Hz, 2H,  $\text{PCH}_2$ ), 4.06 (t,  $J$  = 5.2 Hz, 2H,  $\text{OCH}_2\text{CH}_2\text{N}$ ), 4.06–4.10 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 5.14 (t,  $J$  = 5.2 Hz, 2H,  $\text{CH}_2\text{N}$ ) ppm;  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 150 MHz):  $\delta$  = 15.3 (d,  $J$  = 5.9 Hz,  $\text{CCOP}$ ), 50.3 (s, CN), 62.8 (d,  $J$  = 6.5 Hz,  $\text{CCOP}$ ), 64.0 (d,  $J$  = 164.7 Hz, PC), 71.1 (d,  $J$  = 11.9 Hz,  $\text{PCOC}$ ), 131.0 and 139.0 (2 s, C=C), 159.1 and 163.9 (2 s, C=O) ppm;  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ , 243 MHz):  $\delta$  = 21.3 ppm.

*Diethyl 2-[2-(4,5-dicarbamoyl-1H-1,2,3-triazol-1-yl)ethoxy]ethylphosphonate (9d,  $\text{C}_{12}\text{H}_{22}\text{N}_5\text{O}_6\text{P}$ )*

From 0.264 g diester **8d** (0.671 mmol) diamide **9d** was obtained as a white amorphous solid (0.090 g, 37 %) after chromatography on a silica gel column with chloroform/methanol (50:1, v/v) and crystallisation from ethyl acetate. M.p.: 102–103 °C; IR (KBr):  $\bar{\nu}$  = 3,400, 3,306, 3,214, 2,986, 2,906, 1,672, 1,608, 1,453, 1,245, 1,109, 1,024, 961  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  = 1.31 (t,  $J$  = 6.9 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 2.05 (dt,  $J$  = 15.3, 7.8 Hz, 2H,  $\text{PCH}_2$ ), 3.69 (dt,  $J$  = 11.1, 7.8 Hz, 2H,  $\text{PCH}_2\text{CH}_2\text{O}$ ), 3.91 (t,  $J$  = 5.4 Hz, 2H,  $\text{OCH}_2\text{CH}_2\text{N}$ ), 4.01–4.12 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 5.11 (t,  $J$  = 5.4 Hz, 2H,  $\text{OCH}_2\text{CH}_2\text{N}$ ), 6.00 (s, 1H, NH), 6.07 (s, 1H, NH), 7.60 (s, 1H, NH), 10.77 (s, 1H, NH) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  = 16.6 (d,  $J$  = 6.0 Hz,  $\text{CCOP}$ ), 26.9 (d,  $J$  = 139.1 Hz, PC), 51.0 (s, OCCN), 61.9 (d,  $J$  = 6.3 Hz,  $\text{CCOP}$ ), 65.0 (s, PCCO), 69.1 (s, OCCN), 130.9 and 139.7 (2 s, C=C), 158.7 and 163.7 (2 s, C=O) ppm;  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 121 MHz):  $\delta$  = 29.2 ppm.

*Diethyl 3-(4,5-dicarbamoyl-1H-1,2,3-triazol-1-yl)-2-hydroxypropylphosphonate (9e,  $\text{C}_{11}\text{H}_{20}\text{N}_5\text{O}_6\text{P}$ )*

From 0.145 g diester **8e** (0.382 mmol) diamide **9e** was obtained as a white amorphous solid (0.060 g, 45 %) after chromatography on a silica gel column with chloroform/methanol (50:1, v/v) and crystallisation from methanol. M.p.: 142–143 °C; IR (KBr):  $\bar{\nu}$  = 3,428, 3,308, 3,034, 2,988, 1,686, 1,667, 1,599, 1,443, 1,375, 1,293, 1,113, 1,083, 1,055, 1,029, 960  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 300 MHz):  $\delta$  = 1.32 (t,  $J$  = 7.2 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 2.06 (ddd,  $J$  = 22.8, 15.3, 7.8 Hz, 1H,  $\text{PCH}_a\text{H}_b$ ), 2.18 (ddd,  $J$  = 20.4, 15.3, 5.1 Hz, 1H,  $\text{PCH}_a\text{H}_b$ ), 4.07–4.19 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 4.37–4.50 (m, 1H,  $\text{PCCCH}_a\text{H}_b$ ), 4.99 (dd,  $J$  = 13.5, 8.1 Hz, 1H,  $\text{PCCCH}_a\text{H}_b$ ), 5.02 (dd,  $J$  = 13.5, 4.2 Hz, 1H,  $\text{PCCCH}_a\text{H}_b$ ) ppm;  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 75 MHz):  $\delta$  = 16.9 (d,  $J$  = 6.3 Hz,  $\text{CCOP}$ ), 32.3 (d,  $J$  = 141.7 Hz, PC), 58.1 (d,  $J$  = 18.0 Hz, PCCC), 63.4 and 63.7 (2 d,  $J$  = 6.6 Hz,  $\text{CCOP}$ ), 67.0 (d,  $J$  = 4.0 Hz, PCC), 132.6 and 140.4 (2 s, C=C), 160.6 and 165.3 (2 s, C=O) ppm;  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ , 121 MHz):  $\delta$  = 30.1 ppm.

*Diethyl 3-(4,5-dicarbamoyl-1H-1,2,3-triazol-1-yl)-1-hydroxypropylphosphonate (9f,  $\text{C}_{11}\text{H}_{20}\text{N}_5\text{O}_6\text{P}$ )*

From 0.180 g diester **8f** (0.475 mmol) diamide **9f** was obtained as a white amorphous solid (0.093 g, 56 %) after crystallisation from methanol. M.p.: 179–180 °C; IR (KBr):  $\bar{\nu}$  = 3,433, 3,319, 3,212, 2,974, 2,930, 2,874, 1,668, 1,605, 1,455, 1,399, 1,221, 1,167, 1,066, 1,014, 953  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 300 MHz):  $\delta$  = 1.32 (t,  $J$  = 7.2 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 2.13–2.28 (m, 1H,  $\text{PCCCH}_a\text{H}_b$ ), 2.31–2.44 (m, 1H,  $\text{PCCCH}_a\text{H}_b$ ), 3.89 (ddd,  $J$  = 10.5, 7.2, 3.0 Hz, 1H,  $\text{PCH}$ ), 4.10–4.21 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 4.95–5.14 (m, 2H,  $\text{CH}_2\text{N}$ ) ppm; solubility of **9f** in  $\text{D}_2\text{O}$  or  $\text{CD}_3\text{OD}$  was not sufficient to measure the  $^{13}\text{C}$  NMR spectrum;  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ , 121 MHz):  $\delta$  = 25.4 ppm.

*Diethyl 2-(4,5-dicarbamoyl-1H-1,2,3-triazol-1-yl)-1-hydroxyethylphosphonate (9g,  $\text{C}_{10}\text{H}_{18}\text{N}_5\text{O}_6\text{P}$ )*

From 0.230 g diester **8g** (0.630 mmol) diamide **9g** was obtained as a white amorphous solid (0.112 g, 53 %) after chromatography on a silica gel column with chloroform/methanol (20:1, v/v) and crystallisation from methanol. M.p.: 200–202 °C; IR (KBr):  $\bar{\nu}$  = 3,431, 3,223, 3,081, 2,990, 2,847, 1,671, 1,599, 1,452, 1,393, 1,289, 1,225, 1,031, 979  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 600 MHz):  $\delta$  = 1.39 and 1.40 (2 t,  $J$  = 7.2 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 4.22–4.29 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 4.55 (ddd,  $J$  = 10.2, 9.9 Hz, 3.6 Hz, 1H,  $\text{PCH}$ ), 5.10 (ddd,  $J$  = 13.5, 10.2, 8.7 Hz, 1H,  $\text{PCCCH}_a\text{H}_b$ ), 5.22 (ddd,  $J$  = 13.5, 3.6, 3.1 Hz, 1H,  $\text{PCCCH}_a\text{H}_b$ ) ppm; solubility of **9g** in  $\text{D}_2\text{O}$  and  $\text{CD}_3\text{OD}$  was not sufficient to measure the  $^{13}\text{C}$  NMR spectrum;  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ , 243 MHz):  $\delta$  = 21.2 ppm.

*Synthesis of dihydrazides 10a–10g (general procedure)*

A solution of the diester **8a–8g** (0.73 mmol) and 0.530  $\text{cm}^3$  hydrazine hydrate (10.8 mmol) in 6  $\text{cm}^3$  ethanol was refluxed for 2 h. The reaction mixtures were concentrated to give yellow oils or solids which were subjected to chromatography on a silica gel column with chloroform/methanol or crystallisation to obtain dihydrazides **10a–10g**.

*Diethyl 2-[4,5-bis(hydrazinocarbonyl)-1H-1,2,3-triazol-1-yl]ethylphosphonate (10a,  $\text{C}_{10}\text{H}_{20}\text{N}_7\text{O}_5\text{P}$ )*

From 0.115 g diester **8a** (0.330 mmol) dihydrazide **10a** was obtained (0.070 g, 61 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, 10:1, v/v) as a white amorphous solid. M.p.: 84–86 °C; IR (KBr):  $\bar{\nu}$  = 3,335, 3,282, 2,984, 2,932, 1,661, 1,606, 1,551, 1,491, 1,262, 1,223, 1,056, 1,020, 963  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 300 MHz):  $\delta$  = 1.32 (t,  $J$  = 6.6 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{O}$ ), 2.48–2.59 (m, 2H,  $\text{PCH}_2$ ), 4.07–4.18 (m, 4H,  $\text{CH}_3\text{CH}_2\text{O}$ ), 5.03–5.14 (m, 2H,  $\text{CH}_2\text{N}$ ) ppm;  $^{13}\text{C}$  NMR

(CD<sub>3</sub>OD, 75 MHz):  $\delta$  = 16.9 (d,  $J$  = 6.0 Hz, CCOP), 27.4 (d,  $J$  = 140.0 Hz, PC), 47.1 (s, PCCN), 63.8 (d,  $J$  = 6.3 Hz, CCOP  $\times$  2), 130.8 and 139.3 (2 s, C=C), 157.6 and 161.8 (2 s, C=O) ppm; <sup>31</sup>P NMR (CD<sub>3</sub>OD, 121 MHz):  $\delta$  = 28.2 ppm.

*Diethyl 3-[4,5-bis(hydrazinocarbonyl)-1H-1,2,3-triazol-1-yl]propylphosphonate (10b, C<sub>11</sub>H<sub>22</sub>N<sub>7</sub>O<sub>5</sub>P)*

From 0.170 g diester **8b** (0.468 mmol) dihydrazide **10b** was obtained as a white amorphous solid (0.113 g, 66 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, 10:1, v/v). M.p.: 73–75 °C; IR (KBr):  $\bar{\nu}$  = 3,300, 3,192, 2,983, 2,931, 1,656, 1,551, 1,209, 1,024, 969 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  = 1.28 (t,  $J$  = 6.9 Hz, 6H, CH<sub>3</sub>CH<sub>2</sub>OP), 1.71–1.82 (m, 2H, PCH<sub>2</sub>CH<sub>2</sub>), 2.13–2.27 (m, 2H, PCH<sub>2</sub>), 4.00–4.13 (m, 4H, CH<sub>3</sub>CH<sub>2</sub>OP), 4.94 (t,  $J$  = 7.2 Hz, 2H, CH<sub>2</sub>N), 5.15–6.03 (brs, 6H, NHNH<sub>2</sub>) ppm; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):  $\delta$  = 16.7 (d,  $J$  = 6.0 Hz, CCOP), 22.9 (d,  $J$  = 139.9 Hz, PC), 23.9 (d,  $J$  = 7.4 Hz, PCC), 51.8 (d,  $J$  = 18.9 Hz, PCCC), 62.0 (d,  $J$  = 6.6 Hz, CCOP), 129.5 and 137.6 (2 s, C=C), 156.3 and 161.3 (2 s, C=O) ppm; <sup>31</sup>P NMR (CDCl<sub>3</sub>, 121 MHz):  $\delta$  = 31.5 ppm.

*Diethyl 2-[4,5-bis(hydrazinocarbonyl)-1H-1,2,3-triazol-1-yl]ethoxy]methylphosphonate (10c, C<sub>11</sub>H<sub>22</sub>N<sub>7</sub>O<sub>6</sub>P)*

From 0.430 g diester **8c** (1.13 mmol) dihydrazide **10c** was obtained as a colourless oil (0.139 g, 32 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, 10:1, v/v). IR (film):  $\bar{\nu}$  = 3,326, 3,248, 2,985, 2,918, 2,890, 1,666, 1,544, 1,440, 1,300, 1,238, 1,117, 1,024, 968 cm<sup>-1</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 600 MHz):  $\delta$  = 1.28 (t,  $J$  = 7.0 Hz, 6H, CH<sub>3</sub>CH<sub>2</sub>OP), 3.89 (d,  $J$  = 8.5 Hz, 2H, PCH<sub>2</sub>), 4.05–4.10 (m, 6H, CH<sub>3</sub>CH<sub>2</sub>OP and OCH<sub>2</sub>CH<sub>2</sub>N), 5.14 (t,  $J$  = 4.8 Hz, 2H, OCH<sub>2</sub>CH<sub>2</sub>N) ppm; <sup>13</sup>C NMR (CD<sub>3</sub>OD, 150 MHz):  $\delta$  = 15.3 (d,  $J$  = 5.5 Hz, CCOP), 50.2 (s, CN), 62.8 (d,  $J$  = 6.5 Hz, CCOP), 64.0 (d,  $J$  = 164.9 Hz, PC), 71.1 (d,  $J$  = 11.9 Hz, PCOC), 129.9 and 137.9 (2 s, C=C), 156.5 and 160.5 (2 s, C=O) ppm; <sup>31</sup>P NMR (CD<sub>3</sub>OD, 243 MHz):  $\delta$  = 21.4 ppm.

*Diethyl 2-[2-[4,5-bis(hydrazinocarbonyl)-1H-1,2,3-triazol-1-yl]ethoxy]ethylphosphonate (10d, C<sub>12</sub>H<sub>24</sub>N<sub>7</sub>O<sub>6</sub>P)*

From 0.216 g diester **8d** (0.549 mmol) dihydrazide **10d** was obtained as a white amorphous solid (0.128 g, 59 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, v/v) and crystallisation from ethyl acetate. M.p.: 95–96 °C; IR (KBr):  $\bar{\nu}$  = 3,300, 3,232, 2,983, 2,913, 2,827, 1,667, 1,545, 1,550, 1,252, 1,023, 966, 918 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  = 1.31 (t,  $J$  = 7.2 Hz, 6H, CH<sub>3</sub>CH<sub>2</sub>OP), 2.03 (dt,  $J$  = 15.0, 7.5 Hz, 2H, PCH<sub>2</sub>), 3.69 (dt,  $J$  = 11.1, 7.5 Hz, 2H, PCH<sub>2</sub>CH<sub>2</sub>O), 3.91 (t,  $J$  = 5.7 Hz, 2H, OCH<sub>2</sub>CH<sub>2</sub>N), 4.01–4.13 (m, 4H, CH<sub>3</sub>CH<sub>2</sub>OP), 4.20 (brs, 4H, NH<sub>2</sub>), 5.13 (t,  $J$  = 5.7 Hz, 2H,

OCH<sub>2</sub>CH<sub>2</sub>N), 8.81 (s, 1H, NH), 12.0 (s, 1H, NH) ppm; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):  $\delta$  = 16.6 (d,  $J$  = 6.0 Hz, CCOP), 27.0 (d,  $J$  = 138.5 Hz, PC), 51.0 (s, OCCN), 61.9 (d,  $J$  = 6.3 Hz, CCOP), 65.0 (s, PCCO), 69.1 (s, OCCN), 129.9 and 137.5 (2 s, C=C), 156.5 and 161.3 (2 s, C=O) ppm; <sup>31</sup>P NMR (CDCl<sub>3</sub>, 121 MHz):  $\delta$  = 28.5 ppm.

*Diethyl 3-[4,5-bis(hydrazinocarbonyl)-1H-1,2,3-triazol-1-yl]-2-hydroxypropylphosphonate (10e, C<sub>11</sub>H<sub>22</sub>N<sub>7</sub>O<sub>6</sub>P)*

From 0.290 g diester **8e** (0.764 mmol) dihydrazide **10e** was obtained as a white amorphous solid (0.140 g, 48 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, v/v) and crystallisation from ethanol. M.p.: 140–142 °C; IR (KBr):  $\bar{\nu}$  = 3,336, 3,275, 2,980, 2,930, 1,668, 1,578, 1,549, 1,443, 1,260, 1,217, 1,067, 1,027, 957 cm<sup>-1</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 600 MHz):  $\delta$  = 1.34 and 1.35 (2 t,  $J$  = 7.1 Hz, 6H, CH<sub>3</sub>CH<sub>2</sub>OP), 2.12 (ddd,  $J$  = 23.6, 15.4, 8.2 Hz, 1H, PCH<sub>a</sub>H<sub>b</sub>), 2.18 (ddd,  $J$  = 20.1, 15.4, 4.7 Hz, 1H, PCH<sub>a</sub>H<sub>b</sub>), 4.11–4.19 (m, 4H, CH<sub>3</sub>CH<sub>2</sub>OP), 4.44–4.47 (m, 1H, PCCCH), 4.96 (dd,  $J$  = 13.4, 8.4 Hz, 1H, PCCCH<sub>a</sub>H<sub>b</sub>), 5.05 (dd,  $J$  = 13.4, 4.1 Hz, 1H, PCCCH<sub>a</sub>H<sub>b</sub>) ppm; <sup>13</sup>C NMR (CD<sub>3</sub>OD, 150 MHz):  $\delta$  = 15.3 (d,  $J$  = 5.8 Hz, CCOP), 30.8 (d,  $J$  = 141.6 Hz, PC), 56.4 (d,  $J$  = 17.3 Hz, PCCC), 61.9 and 62.2 (2 d,  $J$  = 6.5 Hz, CCOP), 65.6 (d,  $J$  = 3.6 Hz, PCC), 130.1 and 137.9 (2 s, C=C), 156.7 and 160.6 (2 s, C=O) ppm; <sup>31</sup>P NMR (CD<sub>3</sub>OD, 243 MHz):  $\delta$  = 28.9 ppm.

*Diethyl 3-[4,5-bis(hydrazinocarbonyl)-1H-1,2,3-triazol-1-yl]-1-hydroxypropylphosphonate (10f, C<sub>11</sub>H<sub>22</sub>N<sub>7</sub>O<sub>6</sub>P)*

From 0.262 g diester **8f** (0.691 mmol) dihydrazide **10f** was obtained as a white amorphous solid (0.155 g, 60 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 30:1, v/v). M.p.: 119–120 °C; IR (KBr):  $\bar{\nu}$  = 3,316, 3,266, 3,160, 2,976, 2,930, 1,659, 1,572, 1,532, 1,249, 1,210, 1,072, 1,022, 955 cm<sup>-1</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz):  $\delta$  = 1.31 (t,  $J$  = 7.5 Hz, 6H, CH<sub>3</sub>CH<sub>2</sub>OP), 2.13–2.29 (m, 1H, PCCCH<sub>a</sub>H<sub>b</sub>), 2.31–2.44 (m, 1H, PCCCH<sub>a</sub>H<sub>b</sub>), 3.91 (ddd,  $J$  = 10.8, 7.8, 3.3 Hz, 1H, PCH), 4.09–4.21 (m, 4H, CH<sub>3</sub>CH<sub>2</sub>OP), 4.96–5.15 (m, 2H, CH<sub>2</sub>N) ppm; <sup>13</sup>C NMR (CD<sub>3</sub>OD, 75 MHz):  $\delta$  = 17.0 (d,  $J$  = 5.4 Hz, CCOP), 33.4 (s, PCC), 48.4 (d,  $J$  = 18.9 Hz, PCCC), 64.2 and 64.5 (d,  $J$  = 7.1 Hz, CCOP), 65.6 (d,  $J$  = 166.7 Hz, PC), 130.9 and 139.2 (2 s, C=C), 157.7 and 161.8 (2 s, C=O  $\times$  2) ppm; <sup>31</sup>P NMR (CD<sub>3</sub>OD, 121 MHz):  $\delta$  = 25.5 ppm.

*Diethyl 2-[4,5-bis(hydrazinocarbonyl)-1H-1,2,3-triazol-1-yl]-1-hydroxyethylphosphonate (10g, C<sub>10</sub>H<sub>20</sub>N<sub>7</sub>O<sub>6</sub>P)*

From 0.450 g diester **8g** (1.23 mmol) dihydrazide **10g** was obtained as a white amorphous solid (0.128 g, 28 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 30:1, v/v). M.p.: 149–152 °C; IR (KBr):  $\bar{\nu}$  = 3,334, 3,207, 2,980, 2,930, 2,870, 1,668, 1,578, 1,449,



1,217, 1,067, 1,028, 953  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 600 MHz):  $\delta$  = 1.31 (t,  $J$  = 7.0 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 4.18–4.24 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 4.53–4.58 (m, 1H, PCH), 4.94–5.01 (m, 1H,  $\text{PCCCH}_a\text{H}_b$ ), 5.11–5.16 (m, 1H,  $\text{PCCCH}_a\text{H}_b$ ) ppm;  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , 150 MHz):  $\delta$  = 15.7 (d,  $J$  = 4.6 Hz, CCOP), 51.9 (d,  $J$  = 11.2 Hz, PCCN), 64.7 and 64.8 (2 d,  $J$  = 7.4 Hz, CCOP), 65.5 (d,  $J$  = 165.8 Hz, PC), 130.4 and 137.5 (2 s, C=C), 156.5 and 160.2 (2 s, C=O) ppm;  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ , 243 MHz):  $\delta$  = 21.8 ppm.

*Synthesis of 1,2,3-triazolopyridazinediones 11a–11g (general procedure)*

A mixture of the dihydrazide **10a–10g** (0.50 mmol) and 5  $\text{cm}^3$  10 % hydrochloric acid was heated at 90  $^\circ\text{C}$  for 2.5 h. After concentration in vacuo, crude products were purified on a silica gel column with chloroform/methanol or crystallised from the appropriate solvent.

*Diethyl 2-(4,7-dioxo-5,6-dihydro-1H-1,2,3-triazolo[4,5-d]pyridazin-1-yl)ethylphosphonate (11a,  $\text{C}_{10}\text{H}_{16}\text{N}_5\text{O}_5\text{P}$ )*

From 0.167 g dihydrazide **10a** (0.478 mmol) compound **11a** was obtained as a white amorphous solid (0.045 g, 30 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, 10:1, v/v) followed by crystallisation from ethanol. M.p.: 198–200  $^\circ\text{C}$ ; IR (KBr):  $\bar{\nu}$  = 3,385, 2,922, 2,672, 1,687, 1,582, 1,460, 1,262, 1,214, 1,014, 1,019, 980  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 300 MHz):  $\delta$  = 1.28 (t,  $J$  = 6.6 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 2.58 (dt,  $J$  = 14.7 Hz,  $J$  = 7.5 Hz,  $\text{PCH}_2$ ), 4.03–4.13 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 5.12 (dt,  $J$  = 12.6 Hz,  $J$  = 7.5 Hz,  $\text{CH}_2\text{N}$ ) ppm;  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 150 MHz):  $\delta$  = 17.5 (d,  $J$  = 5.7 Hz, CCOP), 28.2 (d,  $J$  = 141.0 Hz, PC), 46.5 (d,  $J$  = 3.0 Hz, PCCN), 64.7 (d,  $J$  = 6.6 Hz, CCOP), 131.3 and 141.8 (2 s, C=C), 152.0 and 154.6 (2 s, C=O) ppm;  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ , 121 MHz):  $\delta$  = 27.7 ppm.

*Diethyl 3-(4,7-dioxo-5,6-dihydro-1H-1,2,3-triazolo[4,5-d]pyridazin-1-yl)propylphosphonate (11b,  $\text{C}_{11}\text{H}_{18}\text{N}_5\text{O}_5\text{P}$ )*

From 0.055 g dihydrazide **10b** (0.151 mmol) compound **11b** was obtained as a white amorphous solid (0.033 g, 66 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, 10:1, v/v). M.p.: 148–150  $^\circ\text{C}$ ; IR (KBr):  $\bar{\nu}$  = 3,446, 3,074, 2,991, 2,960, 1,662, 1,555, 1,462, 1,294, 1,218, 1,032, 972, 805  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 300 MHz):  $\delta$  = 1.31 (t,  $J$  = 6.9 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 1.84–1.95 (m, 2H,  $\text{PCH}_2\text{CH}_2$ ), 2.24–2.38 (m, 2H,  $\text{PCH}_2$ ), 4.02–4.15 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 4.93 (t,  $J$  = 6.9 Hz, 2H,  $\text{CH}_2\text{N}$ ) ppm;  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 75 MHz):  $\delta$  = 16.7 (d,  $J$  = 5.7 Hz, CCOP), 22.7 (d,  $J$  = 141.9 Hz, PC), 23.9 (s, PCC), 50.5 (d,  $J$  = 18.5 Hz, PCCC), 62.6 (d,  $J$  = 6.5 Hz, CCOP), 129.1 and 139.7 (2 s,

C=C), 150.5 and 152.7 (2 s, C=O) ppm;  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ , 121 MHz):  $\delta$  = 32.6 ppm.

*Diethyl [2-(4,7-dioxo-5,6-dihydro-1H-1,2,3-triazolo[4,5-d]pyridazin-1-yl)ethoxy]methylphosphonate (11c,  $\text{C}_{11}\text{H}_{18}\text{N}_5\text{O}_6\text{P}$ )*

From 0.090 g dihydrazide **10c** (0.237 mmol) compound **11c** was obtained as a white amorphous solid (0.043 g, 52 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, v/v). M.p.: 170–172  $^\circ\text{C}$ ; IR (KBr):  $\bar{\nu}$  = 3,348, 2,985, 2,930, 2,918, 1,658, 1,552, 1,420, 1,260, 1,070, 1,028, 975  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 600 MHz):  $\delta$  = 1.25 (t,  $J$  = 7.0 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 3.89 (d,  $J$  = 8.5 Hz, 2H,  $\text{PCH}_2$ ), 4.01–4.04 (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 4.16 (t,  $J$  = 5.1 Hz, 2H,  $\text{OCH}_2\text{CH}_2\text{N}$ ), 5.09 (t,  $J$  = 5.1 Hz, 2H,  $\text{OCH}_2\text{CH}_2\text{N}$ ) ppm;  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 150 MHz):  $\delta$  = 15.2 (d,  $J$  = 5.7 Hz, CCOP), 49.2 (s, CN), 62.7 (d,  $J$  = 6.6 Hz, CCOP), 63.9 (d,  $J$  = 164.8 Hz, PC), 70.9 (d,  $J$  = 12.1 Hz, PCOC), 129.2 and 139.3 (2 s, C=C), 149.6 and 152.3 (2 s, C=O) ppm;  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ , 243 MHz):  $\delta$  = 21.2 ppm.

*Diethyl 2-[2-(4,7-dioxo-5,6-dihydro-1H-1,2,3-triazolo[4,5-d]pyridazin-1-yl)ethoxy]ethylphosphonate (11d,  $\text{C}_{12}\text{H}_{20}\text{N}_5\text{O}_6\text{P}$ )*

From 0.165 g dihydrazide **10d** (0.419 mmol) compound **11d** was obtained as a white amorphous solid (0.087 g, 58 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, v/v) and crystallisation from ethyl acetate/chloroform. M.p.: 134–135  $^\circ\text{C}$ ; IR (KBr):  $\bar{\nu}$  = 3,433, 2,974, 2,928, 2,872, 1,715, 1,645, 1,206, 1,113, 1,065, 1,026, 982, 952  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  = 1.26 (t,  $J$  = 7.2 Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ), 2.07 (dt,  $J$  = 15.0, 7.2 Hz, 2H,  $\text{PCH}_2$ ), 3.73 (dt,  $J$  = 13.5, 7.5 Hz, 2H,  $\text{PCH}_2\text{CH}_2\text{O}$ ), 3.96–4.10 (m, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$  and  $\text{OCH}_2\text{CH}_2\text{N}$ ), 5.01 (t,  $J$  = 5.1 Hz, 2H,  $\text{OCH}_2\text{CH}_2\text{N}$ ), 10.49 (brs, 2H, NH) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta$  = 16.6 (d,  $J$  = 6.3 Hz, CCOP), 26.8 (d,  $J$  = 138.9 Hz, PC), 49.9 (s, OCCN), 62.2 (d,  $J$  = 6.5 Hz, CCOP), 65.0 (s, PCCO), 68.8 (s, OCCN), 129.4 and 139.4 (2 s, C=C), 151.1 and 152.8 (2 s, C=O) ppm;  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ , 121 MHz):  $\delta$  = 29.3 ppm.

*Diethyl 3-(4,7-dioxo-5,6-dihydro-1H-1,2,3-triazolo[4,5-d]pyridazin-1-yl)-2-hydroxypropylphosphonate (11e,  $\text{C}_{11}\text{H}_{18}\text{N}_5\text{O}_6\text{P}$ )*

From 0.270 g dihydrazide **10e** (0.719 mmol) compound **11e** was obtained as a white amorphous solid (0.087 g, 39 %) after chromatography on a silica gel column with chloroform/methanol (20:1, 10:1, v/v) and crystallisation from water. M.p.: 168–170  $^\circ\text{C}$ ; IR (KBr):  $\bar{\nu}$  = 3,476, 2,982, 2,932, 2,867, 1,655, 1,550, 1,427, 1,405, 1,256, 1,222, 1,108, 1,054, 957  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 600 MHz):

$\delta = 1.30$  and  $1.31$  (2 t,  $J = 6.1$  Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ),  $2.25$  (td,  $J = 16.1$ ,  $8.8$  Hz, 1H,  $\text{PCH}_a\text{H}_b$ ),  $2.35$  (td,  $J = 15.8$ ,  $3.2$  Hz, 1H,  $\text{PCH}_a\text{H}_b$ ),  $4.10$ – $4.17$  (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ),  $4.52$ – $4.59$  (m, 1H,  $\text{PCCCH}_a\text{H}_b$ ),  $4.91$  (dd,  $J = 12.8$ ,  $9.2$  Hz, 1H,  $\text{PCCCH}_a\text{H}_b$ ),  $4.98$  (dd,  $J = 12.8$ ,  $2.6$  Hz, 1H,  $\text{PCCCH}_a\text{H}_b$ ) ppm;  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ ,  $150$  MHz):  $\delta = 15.6$  (d,  $J = 5.7$  Hz,  $\text{CCOP}$ ),  $30.0$  (d,  $J = 139.9$  Hz,  $\text{PC}$ ),  $55.7$  (d,  $J = 17.8$  Hz,  $\text{PCCC}$ ),  $63.5$  and  $63.6$  (2 d,  $J = 6.4$  Hz,  $\text{CCOP}$ ),  $65.4$  (d,  $J = 3.8$  Hz,  $\text{PCC}$ ),  $130.1$  and  $140.1$  (2 s,  $\text{C}=\text{C}$ ),  $149.8$  and  $153.4$  (2 s,  $\text{C}=\text{O}$ ) ppm;  $^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ ,  $243$  MHz):  $\delta = 30.3$  ppm.

*Diethyl 3-(4,7-dioxo-5,6-dihydro-1H-1,2,3-triazolo-[4,5-d]pyridazin-1-yl)-1-hydroxypropylphosphonate (11f,  $\text{C}_{11}\text{H}_{18}\text{N}_5\text{O}_6\text{P}$ )*

From  $0.163$  g dihydrazide **10f** ( $0.430$  mmol) compound **11f** was obtained as a white amorphous solid ( $0.086$  g,  $58$  %) after chromatography on a silica gel column with chloroform/methanol ( $50:1$ ,  $20:1$ ,  $10:1$ , v/v). M.p.:  $66$ – $68$  °C; IR (KBr):  $\bar{\nu} = 3,362$ ,  $2,987$ ,  $2,920$ ,  $1,666$ ,  $1,580$ ,  $1,449$ ,  $1,212$ ,  $1,050$ ,  $1,021$ ,  $970$   $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ,  $300$  MHz):  $\delta = 1.32$  (t,  $J = 7.2$  Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ),  $2.19$ – $2.28$  (m, 1H,  $\text{PCCCH}_a\text{H}_b$ ),  $2.31$ – $2.44$  (m, 1H,  $\text{PCCCH}_a\text{H}_b$ ),  $3.89$  (ddd,  $J = 10.5$ ,  $7.2$ ,  $3.0$  Hz, 1H,  $\text{PCH}$ ),  $4.10$ – $4.21$  (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ),  $4.95$ – $5.12$  (m, 2H,  $\text{CH}_2\text{N}$ ) ppm;  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ,  $75$  MHz):  $\delta = 17.0$  (d,  $J = 5.4$  Hz,  $\text{CCOP}$ ),  $33.3$  (d,  $J = 4.0$  Hz,  $\text{PCC}$ ),  $48.1$  (d,  $J = 16.2$  Hz,  $\text{PCCC}$ ),  $64.2$  and  $64.5$  (d,  $J = 6.9$  Hz,  $\text{CCOP}$ ),  $65.4$  (d,  $J = 167.3$  Hz,  $\text{PC}$ ),  $130.2$  and  $140.8$  (2 s,  $\text{C}=\text{C}$ ),  $148.9$  and  $152.2$  (2 s,  $\text{C}=\text{O}$ ) ppm;  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ ,  $121$  MHz):  $\delta = 25.3$  ppm.

*Diethyl 2-(4,7-dioxo-5,6-dihydro-1H-1,2,3-triazolo-[4,5-d]pyridazin-1-yl)-1-hydroxyethylphosphonate (11g,  $\text{C}_{10}\text{H}_{16}\text{N}_5\text{O}_6\text{P}$ )*

From  $0.100$  g dihydrazide **10g** ( $0.274$  mmol) compound **11g** was obtained as a colourless oil ( $0.036$  g,  $40$  %) after chromatography on a silica gel column with chloroform/methanol ( $50:1$ ,  $10:1$ , v/v). IR (film):  $\bar{\nu} = 3,362$ ,  $2,988$ ,  $2,971$ ,  $1,667$ ,  $1,580$ ,  $1,212$ ,  $1,021$   $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ,  $600$  MHz):  $\delta = 1.38$  and  $1.39$  (2 t,  $J = 7.0$  Hz, 6H,  $\text{CH}_3\text{CH}_2\text{OP}$ ),  $4.23$ – $4.29$  (m, 4H,  $\text{CH}_3\text{CH}_2\text{OP}$ ),  $4.64$ – $4.69$  (m, 1H,  $\text{PCH}$ ),  $5.09$ – $5.15$  (m, 2H,  $\text{CH}_2\text{N}$ ) ppm;  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ,  $150$  MHz):  $\delta = 15.3$  and  $15.4$  (d,  $J = 5.2$  Hz,  $\text{CCOP}$ ),  $51.3$  (d,  $J = 11.6$  Hz,  $\text{PCCN}$ ),  $63.3$  and  $63.5$  (2 d,  $J = 6.8$  Hz,  $\text{COP}$ ),  $66.4$  (d,  $J = 166.2$  Hz,  $\text{PC}$ ),  $129.2$  and  $139.4$  (2 s,  $\text{C}=\text{C}$ ),  $150.1$  and  $152.4$  (2 s,  $\text{C}=\text{O}$ ) ppm;  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ ,  $243$  MHz):  $\delta = 20.5$  ppm.

*Assays for antiviral activity other than HIV*

The compounds were evaluated against the following viruses: herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient ( $\text{TK}^-$ ) HSV-1 KOS strain

resistant to ACV ( $\text{ACV}^r$ ), herpes simplex virus type 2 (HSV-2) strain G, cytomegalovirus (strains AD-169 and Davis), varicella-zoster virus (VZV) (strains OKA and YS), vaccinia virus Lederle strain, respiratory syncytial virus (RSV) strain Long, vesicular stomatitis virus (VSV), coxsackie b4 virus, parainfluenza 3, influenza virus A (subtypes H1N1, H3N2), influenza virus B, reovirus-1, Sindbis virus and Punta Toro virus. The antiviral assays were based on inhibition of virus-induced cytopathicity or plaque formation in human embryonic lung (HEL) fibroblasts, African green monkey cells (Vero), human epithelial cervix carcinoma cells (HeLa) or MDCK. Confluent cell cultures in microtiter 96-well plates were inoculated with  $100$  CCID<sub>50</sub> of virus ( $1$  CCID<sub>50</sub> being the virus dose to infect  $50$  % of the cell cultures) or  $20$  or  $100$  plaque forming units (PFU) (for VZV and CMV, respectively) in the presence of varying concentrations of the test compounds. Viral cytopathicity or plaque formation was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC<sub>50</sub> or compound concentration required to reduce virus-induced cytopathogenicity or viral plaque formation by  $50$  %.

*Anti-HIV activity assays*

Inhibition of HIV-1(III<sub>B</sub>)- and HIV-2(ROD)-induced cytopathicity in CEM cell cultures was measured in microtiter 96-well plates containing  $3 \times 10^5$  CEM cells/ $\text{cm}^3$  infected with  $100$  CCID<sub>50</sub> of HIV per  $\text{cm}^3$  and containing appropriate dilutions of the test compounds. After  $4$ – $5$  days of incubation at  $37$  °C in a  $\text{CO}_2$ -controlled humidified atmosphere, CEM giant cell (syncytium) formation was examined microscopically. The EC<sub>50</sub> ( $50$  % effective concentration) was defined as the compound concentration required to inhibit HIV-induced giant cell formation by  $50$  %.

*Cytostatic activity assays*

All assays were performed in 96-well microtiter plates. To each well were added  $(5$ – $7.5) \times 10^4$  tumour cells and a given amount of the test compound. The cells were allowed to proliferate for  $48$  h (murine leukemia L1210 cells) or  $72$  h (human lymphocytic CEM and human cervix carcinoma HeLa cells) at  $37$  °C in a humidified  $\text{CO}_2$ -controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter. The IC<sub>50</sub> ( $50$  % inhibitory concentration) was defined as the concentration of the compound that inhibited cell proliferation by  $50$  %.

**Acknowledgments** The authors are grateful to Leen Ingels, Leentje Persoons, Frieda De Meyer, Lizette van Berckelaer, Anita Camps, Steven Carmans and Lies Van den Heurck for excellent technical assistance. The work was supported by the KU Leuven (GOA 10/14) and by the Medical University of Lodz (502-34-009 and 503/3-014-01/503-1).

**Open Access** This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

## References

1. De Clercq E (2007) *Antiviral Res* 75:1
2. De Clercq E, Holý A (2005) *Nat Rev Drug Disc* 4:928
3. Naesens L, Snoeck R, Andrei G, Balzarini J, Neyts J, De Clercq E (1997) *Antivir Chem Chemother* 8:1
4. Starret JE Jr, Tortolani DR, Hitchcock MJ, Martin JC, Mansuri MM (1992) *Antiviral Res* 19:1484
5. De Clercq E (2003) *Clin Microbiol Rev* 16:569
6. Chen HM, Hosmane RH (2001) *Nucleosides Nucleotides Nucleic Acids* 8:1599
7. Baszczyński O, Jansa P, Dračinsky M, Klepetářová B, Holý A, Votruba I, De Clercq E, Balzarini J, Janeba Z (2011) *Bioorg Med Chem* 19:2114
8. De Clercq E, Holý A, Rosenberg I, Sakuma T, Balzarini J, Maudgal PC (1986) *Nature* 323:464
9. Holý A, Votruba I, Masojdková M, Andrei G, Snoeck R, Naesens L, De Clercq E, Balzarini J (2002) *J Med Chem* 45:1918
10. Hocková D, Holý A, Masojdková M, Andrei G, Snoeck R, De Clercq E, Balzarini J (2002) *J Med Chem* 46:5064
11. Balzarini J, Pannecouque C, Naesens L, Andrei G, Snoeck R, De Clercq E, Hocková D, Holý A (2004) *Nucleosides Nucleotides Nucleic Acids* 23:1321
12. Maag D, Castro C, Hong Z, Cameron CE (2001) *J Biol Chem* 276:46094
13. Wang XL, Wan K, Zhou CH (2010) *Eur J Med Chem* 45:4631
14. Weide T, Saldanha SA, Minond D, Spicer TP, Fotsing JR, Spargaren M, Frere JM, Bebrone C, Sharpless KB, Hodder PS, Fokin VV (2010) *Med Chem Lett* 1:150
15. Chandrika PM, Yakaiah T, Gayatri G, Pranay Kumar K, Narsaiah B, Murthy USN, Raghu Ram Rao A (2010) *Eur J Med Chem* 45:78
16. Aher NG, Pore VS, Mishra NN, Kumar A, Shukla PK, Sharma A, Bhat MK (2009) *Bioorg Med Chem Lett* 19:759
17. Chaudhary PM, Chavan SR, Shirazi F, Razdan M, Nimkar P, Maybhate SP, Likhite AP, Gonnade R, Hazara BG, Deshpande MV, Deshpande SR (2009) *Bioorg Med Chem* 17:2433
18. Stefely JA, Palchaudhuri R, Miller PA, Peterson RJ, Moraski GC, Hergenrother PJ, Miller MJ (2010) *J Med Chem* 53:3389
19. Park SM, Yang H, Park SK, Kim HM, Kim BH (2010) *Bioorg Med Chem Lett* 20:5831
20. Odlo K, Fournier-Dit-Chabert J, Ducki S, Gani OABSM, Sylte I, Hansen TV (2010) *Bioorg Med Chem* 18:6874
21. Wu J, Green N, Hotchandani R, Hu Y, Condon J, Huang A, Kaila N, Li HQ, Guler S, Li W, Tam SY, Wang Q, Pelker J, Marusic S, Hsu S, Hall JP, Telliez JP, Cui J, Lin LL (2009) *Bioorg Med Chem Lett* 19:3485
22. Yoon J, Cho L, Lee SK, Ryu JS (2011) *Bioorg Med Chem Lett* 21:1953
23. Velázquez S, Alvarez R, Pérez C, De Clercq E, Balzarini J, Camarasa MJ (1998) *Antivir Chem Chemother* 9:481
24. Joradão AK, Ferreira VF, Souza TML, De Souza Faria GG, Machado V, Abrantes JL, De Souza MCBV, Cunha AC (2011) *Bioorg Med Chem* 19:1860
25. Montagu A, Roy V, Balzarini J, Snoeck R, Andrei G, Agrofoglio LA (2011) *Eur J Med Chem* 46:778
26. Da Silva FC, De Souza MCBV, Frugulhetti IIP, Castro HC, De Souza SL, De Souza TML, Rodrigues DQ, Souza AMT, Abreu PA, Passamani F, Rodrigues RC, Ferreira VF (2009) *Eur J Med Chem* 44:373
27. Saito Y, Escuret V, Durantel D, Zoulim F, Schinazi RF, Agrofoglio LA (2003) *Bioorg Med Chem* 11:3633
28. Elayadi H, Smietana M, Pannecouque C, Leyssen P, Neyts J, Vasseur JJ, Lazrek HB (2010) *Bioorg Med Chem* 20:7365
29. Borowski P, Lang M, Haag A, Schmitz H, Choe J, Chen HM, Hosmane RS (2002) *Antimicrob Agents Chemother* 46:1231
30. Głowacka IE, Cieślak M, Piotrowska DG (2011) *Phosphorus Sulfur Silicon Relat Elem* 186:431
31. Palacios F, Ochoa de Retana AM, Pagalday J (1994) *Heterocycles* 38:95
32. Głowacka IE, Balzarini J, Wróblewski AE (2012) *Nucleosides Nucleotides Nucleic Acids* 31:293
33. Eger K, Klünder EM, Schmidt M (1994) *J Med Chem* 37:3057
34. Koszytkowska-Stawińska M, De Clercq E, Balzarini J (2009) *Bioorg Med Chem* 17:3756
35. Głowacka IE, Balzarini J, Wróblewski AE (2013) *Arch Pharm Chem Life Sci* 346:278
36. Głowacka IE (2009) *Tetrahedron Asymmetry* 20:2270
37. Abramov VS (1952) *Zh Obshch Khim* 22:647
38. Abramov VS (1953) *Chem Abstr* 47:5351e
39. Davies AJ, Donald ASR, Marks RE (1967) *J Chem Soc C* 1967:2109
40. Wang Y, Inguaggiato G, Jasamai M, Shah M, Hughes D, Slater M, Simons C (1999) *Bioorg Med Chem* 7:481
41. Biagi G, Ciambone F, Livi O, Scartoni V (2002) *J Heterocycl Chem* 39:889
42. Karplus M (1963) *J Am Chem Soc* 85:2870
43. Beneza C (1973) *J Am Chem Soc* 95:6890
44. Neeser JR, Tronchet JMJ, Charollais EJ (1983) *Can J Chem* 61:2112
45. Adiwidjaja G, Meyer B, Thiem J (1979) *Z Naturforsch* 34b:1547
46. Buchanan GW, Bourque K, Seeley A (1986) *Magn Res Chem* 24:360